STUDIES ON SOME INTEGUMENTAL PROTEINS OF INSECTS

BY

HERBERT HUGHES MOOREFIELD

B.S., University of Maryland, 1951
M.S., University of Illinois, 1952

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENTOMOLOGY
IN THE GRADUATE COLLEGE OF THE
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PART ONE: THE CHARACTER AND ULTIMATE FATE OF THE SALIVARY SECRETION OF *PHORMIA REGINA* MEIG. (DIPTERA, CALLIPHORIDAE).

In a comparative, chromatographic study of the material in the late larval salivary glands, and the substance which is responsible for adhering the puparia of *Phormia regina* to their substrate, evidence is presented which indicates that these substances are identical in nature and composition; further, that this "puparial cement" is the ultimate fate of the salivary secretion.

The identity of the secretions, collected from two different sites (the salivary glands and the exterior of the puparium), has been demonstrated by like nitrogen composition; the presence of a single free amino acid, lysine, in each fluid; the same components in the protein constituent (amino acids-glycine, alanine, serine, threonine, valine, leucine, tyrosine, phenylalanine, proline, aspartic acid, glutamic acid, arginine, lysine, cystine, B-aminoisobutyric acid, and the carbohydrate, glucosamine) ; as well as by the reaction to several biochemical characterization tests.

PART TWO: THE NATURE OF ARTHROPODIN.

The untanned larval cuticle of *Sarcophaga crassipalpis* was shown to contain 10.1% nitrogen. The percentage composition was determined as follows: water 55%; dry weight-protein 50.6%, chitin 48.4% ether soluble lipids 0.9%, and ash 0.2%.

Ultracentrifugation of the soluble protein fraction showed a monodisperse system with a calculated molecular weight in the range of 7,500.

Electrophoresis studies proved that arthropodin is not a homogeneous material. At pH 7.5, in a 0.1 ionic phosphate

buffer, cold water extracts of the soluble cuticular protein from *Sarcophaga* consistently demonstrated four components. The velocity and percentage of the components in this water-soluble material, and in a borate buffer extracted fraction are compared.

A polyphenol oxidase was shown to be one of the constituents of arthropodin. Evidence was presented assigning the enzymatic activity to a specific portion of the electrophoretic pattern. This was accomplished by: 1) concentration of the enzyme through ammonium sulfate fractionation; 2) isolation of the activity with a serial fractionation relative to time; and 3) testing specific samples withdrawn directly from the Tiselius apparatus.

Preliminary data on comparative electrophoretic studies of arthropodin from other representative insects is also presented. These indicate that the soluble protein from cuticles of different species are not comparable in the number or relative proportions of components.

Arthropodin was originally defined in terms of its solubility characteristics, but in view of the diversity of previous methods used in its preparation and the presented data relative to its nature, it is suggested that future reference to the term "arthropodin" be restricted to cold water extracts of cuticle; bearing in mind the connotation of a mixed, heterogeneous, protein fraction. Future trends for investigation of soluble cuticular proteins are suggested.

PART THREE: A COMPARATIVE ANALYSIS OF THE FREE AMINO ACID COMPOSITION OF TANNED AND UNTANNED CUTICLE IN *SARCOPHAGA CRASSIPALPIS* MACQ.

The free amino acids of the untanned larval cuticle and

the tanned puparium of *Sarcophaga crassipalpis* were analyzed by paper partition chromatography. Leucine, valine, alanine, glycine, serine, glutamic acid, aspartic acid, and an unidentified polypeptide were common to both. Arginine, lysine, threonine, and an unknown ninhydrin-yellow substance were present only in the puparial preparations, whereas a spot representing B-alanine, glutamine or citrulline was characteristically present in larval skin extracts.

Tryptophane and tyrosine were not present as free amino acids in either type of cuticle, contrary to previous literature reports. This fact lends support to the view that tyrosine is altered structurally before passing from the haemolymph into the cuticle, prior to the tanning process.

VITA

Herbert Hughes Moorefield was born in Baltimore, Maryland, on July 25, 1918. He attended grate, grammer and high school in this city and was graduated from the latter, Baltimore City College, in 1936.

Post high school employment consisted principally of laboratory situations in which he gained experience of a diverse, scientific nature. From 1938-1940, he was employed by the Johns Hopkins Medical School, Department of Pharmacology and Experimental Therapeutics, as a laboratory assistant and was engaged in a research program on the then new sulfonamide drugs. From 1940-1041, he worked for the Bethlehem Steel Corporation as an analyst, performing routine steel and slag control analyses.

During the period from 1936-1941, he was actively engaged in insect study as an avocation, belonged to the Maryland Academy of Sciences, the Natural History Society of Maryland, and served as Curator of the Department of Entomology at the Natural History Society for about four years.

In May 1941, he was called into the U.S. Army and after basic training, was sent to a medical laboratory technician school at Letterman General Hospital, San Francisco. After completion of these studies, he served a short period in a hospital at Moffett Field and was later transferred to a new air base at Chico, California where he established and was placed in charge of the clinical laboratory for a two hundred bed hospital. He remained at this post until his discharge in December, 1945.

After the war, he worked in the engineering research laboratory of the Friez-Bendix Corporation for half a year on a problem concerning the development of a temperature-responsive, electrical resistor. His next position was one in civil service, employed at Camp Detrick, the Army's biological warfare research center, where he was assigned to a project engaged in work on

botulinus toxin. In the summer of 1948, he was granted leave of absence to attend the Johns Hopkins University to study chemistry; he resigned the position in the fall of that year to attend the University of Maryland.

By attending summer classes at the George Washington University and the University of Maryland, he was able to complete his undergraduate training, majoring in entomology, in two and one-half years; received a B.S. degree, with honors, from the University of Maryland in February 1951. He then entered the graduate college, University of Illinois, and completed his M.S. in entomology in February, 1952. He was the recipient of a second and third year University Fellowship at the University of Illinois, but relinquished the latter to accept a research assistantship in insect toxicology studies. Additional entomological experience was gained through one summer's employment at the Maryland Agricultural Experiment Station, screening insecticides; a teaching assistantship at the University of Illinois for one summer, teaching insect taxonomy; and instructing a laboratory section in insect physiology for one semester.

He is now a member of the American Association of Economic Entomologists, the American Mosquito Control Association, the Masonic Order, Phi Kappa Phi, Phi Sigma, Sigma Xi (associate member) and Gamma Alpha.

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